PHYSICAL AND CHEMICAL PROPERTIES OF LACTOSE

The primary carbohydrate of the milk of most mammals is lactose (4-O-β-D-galactopyranosyl-D-glucopyranose), commonly called milk sugar, and milk is the sole source of lactose for all practical purposes. However, the California sea lion and other Pacific pinnipeds have no lactose in their milks (Pilson and Kelly, 1962; Pilson, 1965; Johnson et al. 1974; Stewart et al., 1983). Milks of monotremes, such as the echidna and platypus, contain less than 0.1% lactose (Morrissey. 1985), while human milk contains one of the highest levels of lactose at about 7% (Renner, 1983). Bovine milks average 4.8% anhydrous lactose. amounting to about 50% of the total solids of skimmed milk.

Small amounts of other carbohydrates occur in milk, partly bound to phosphate, lipids and/or proteins and partly free. Cows' milk contains monosaccharides (glucose and galactose) at concentrations of about 10 mg l^{-1} and oligosaccharides, about 100 mg l^{-1} (Renner. 1983). In some milks, other carbohydrates are present at higher concentrations than lactose (Jenness et al., 1964).

Other sources of lactose are rare, e.g. *Forsythia* flowers (Kuhn and Low, 1949). *Sapotacea* (Reithel and Venkataraman. 1956). and as a constituent of some oligosaccharides (Trucco et al., 1954). The reader is referred to the review by Jenness and Sloan (1970) for a discussion of the evolutionary biochemistry of lactose. The literature on lactose is voluminous and several general and specialized reviews are available (Short. 1978; MacBean. 1979;

Paige and Bayless, 1981; Doner and Hicks, 1982; Delmont, 1983; Renner, 1983; Hobman, 1984; Zadow, 1984; Morrissey, 1985; Holsinger, 1987; IDF, 1993). The discussion in this chapter will be concerned with the physical and physico-chemical properties of lactose and their significance in the production and quality of dairy foods and ingredients.

1.2 BIOSYNTHESIS

The biosynthesis of lactose in the mammary gland has been well studied and the literature reviewed (Ebner and Schanbacher, 1974; Jones, 1978). The somewhat unusual biosynthetic pathway involves a whey protein, α-lactalbumin (α-la), acting as a protein modifier of an enzyme, a galactosyltransferase (EC 2.4.1.22). Although the usual physiological function of this enzyme is to transfer galactose to an N-acetylglucosaminyl residue, it may also transfer galactose to glucose in the presence of α-la to form the disaccharide, lactose. The synthesis of α-la is unique to the mammary gland, so, presumably, it is under hormonal control (Ebner and Schanbacher, 1974). The general pathway for lactose synthesis described by Jones (1978) begins with the conversion of one molecule of glucose to UDP-glucose (through glucose-1-P and glucose-6-P) which is epimerized to UDP-galactose. In the presence of galactosyltransferase and α-la, UDP-galactose is condensed with another molecule of glucose to form lactose and UDP. This final step, in which the enzyme is modified by α-la, occurs, not in the cytosol, but in the Golgi vesicles. α-La dissociates from the galactosyltransferase after lactose formation but before it is discharged into the alveolar lumen from the secretory vesicles along with the milk proteins.

Knowledge of the detailed interactions between α-la and galactosyltransferase at the molecular level must be obtained before the question as to how the whey protein modifies the enzyme specifically toward glucose over N-acetylglucosamine can be answered. Berliner and Johnson (1988) point out that two approaches have been taken: structure/function studies with analogues of substrates and inhibitors and physical correlations of active site properties and chemical cross-linking of an active enzyme: α-la complex. Brew and his colleagues (Brew et al., 1975; Richardson and Brew, 1980) showed, using bifunctional lysine-specific cross-linking agents, that the cross-linked lactose synthase complex was fully active and identical, kinetically, to the native complex. They reported that four modified Lys residues in α-la were directly cross-linked to galactosyltransferase. Their later studies, using differential kinetic labelling, showed that Lys 5 and Lys 114 of α-la were affected by complex formation, Lys 114 actually **increasing** in reactivity (emphasis after Berliner). Berliner and Johnson (1988) point out that the detailed metal ion-binding properties of α-la were not known to Brew et al. (1975). The discovery of specific cation binding by α-la was

first reported by Berliner et al. (1978, 1987). More recent studies, reviewed by Berliner and Johnson (1988), have shown that, in addition to its unique calcium-binding properties, α-la binds other cations: some, such as Mn^{2-}, bind preferentially to the calcium-binding site. Further, Murakami and Berliner (1983) reported very strong binding of Zn^{2-}, Co^{2-}, Cu^{2-} and Al^{3+}; when using zinc as an activator of galactosyltransferase (when zinc simultaneously binds to α-la), the lactose synthase activity of apo- or Ca^{2-} α-la were identical in V_{max} and K_m app for α-la. It was not until Berliner et al. (1984) identified a unique hydrophobic binding site in galactosyl-transferase for 2-N-acylglucosamine substituents that physically overlapped the α-la-binding site that the importance of zinc modulation of the conformation of α-la became obvious.

With the development of techniques for genetic manipulation, possibilities for closer investigation of the function of α-la in lactogenesis have opened up. Kumar et al. (1994) have generated mice deficient for β-casein by gene targeting in embryonic stem cells. Although the absence of β-casein expression reduced the total protein concentration in the milk, there was a significant increase in the concentration of the whey proteins that partially compensated for the total protein decrease (Table 1.1). These authors did not report on the concentration of the other milk constituents or concentrations of the individual proteins other than β-casein. Since the growth of pups feeding on the milk from homozygous mutant mice was reduced relative to those feeding on the milk of wild-type mice, other compositional alterations may have occurred.

Stinnakre et al. (1994) created α-la-deficient mice by disrupting the gene by homologous recombination in embryonic stem cells. In this case, although homozygous mutant females were fertile and their offspring viable, the pups could not feed. The mammary gland epithelial cell

TABLE 1.1
Composition milk from genetically manipulated mice

	Wild	Heterozygous	Homozygous
β-Casein negative[a]			
Total protein (mg ml⁻¹)	97.1		87.3
Whey protein (mg ml⁻¹)	18.5		22.0
β-casein (mg ml⁻¹)	21		0
α-Lactalbumin negative[b]			
Total protein (mg ml⁻¹)	83	124	180
α-Lactalbumin (μg ml⁻¹)	500	300	0
Total solids (mg ml⁻¹)	21		611
Viscosity (mPa)	74	235	3224

[a]Kumar et al. (1994).
[b]Stinnakre et al. (1994).

structure appeared normal in these mice, in contrast to a report that α-la inhibits the growth of mammary epithelial cells in culture (Thompson *et al.*, 1992). The milk was devoid of α-la and lactose, rich in protein and fat, highly viscous and apparently could not be expressed from the mammary gland (Table 1.1). Offspring of heterozygous mice could nurse normally without growth retardation; there was a 40% decrease in α-la but only a 10–20% decrease in the lactose content of the milk. These authors also found a negative correlation between fat and α-la concentration and between total protein and α-lactalbumin concentration (Table 1.1), corroborating the relationship among protein, fat and lactose concentrations (Mepham, 1987) and actually reflecting the relationship between lactose and protein concentrations (Mepham, 1987). However, Stinnakre *et al.* (1994) point out that their results suggest that α-la is in excess for lactose synthesis after the onset of lactogenesis since the observed decrease in the lactose content of the milk of their heterozygotes was not proportional to the decrease in α-la. The work of these scientists clearly demonstrates the key function of α-la and its relationship among the milk constituents; additional studies are needed to examine the metabolic alterations further.

Lactose is the principal milk component that affects osmotic pressure; other contributors are the diffusable ions, especially K^+, Na^+ and Cl^-, and other sugars. Since lactose is carried to the lumen of the alveolus in secretory vesicles after synthesis in the Golgi apparatus, water is undoubtedly drawn in osmotically to dilute the sugar (Linzell and Peaker, 1971). The high total solids content and concomitant high viscosity reported for the milks of transgenic mice (Table 1.1) (Stinnakre *et al.*, 1994) strongly suggest that water is necessary to dilute all the milk constituents to reduce the viscosity sufficiently to permit the young to suckle. The composition of the aqueous phase of milk, in terms of lactose, water and the monovalent cations, is relatively constant at 'normal' rates of lactose synthesis (Peaker, 1980); for this reason, the chloride/lactose ratio in milk is used to diagnose udder infections, e.g. mastitis, since lactose and chloride concentrations are complementary (Barry and Rowland, 1953) and infection disrupts the mammary secretory epithelium (Linzell and Peaker, 1971).

Jenness and Sloan (1970) proposed that the synthesis of a disaccharide is evolutionarily advantageous since a given weight of lactose exerts only half the osmotic pressure of the same weight of a monosaccharide; therefore, double the calories can be provided for a given osmotic increment.

1.3 PHYSICAL PROPERTIES

In manufactured products, lactose may occur in either of two crystalline forms, α-hydrate or anhydrous β-, or as an amorphous 'glassy' mixture of

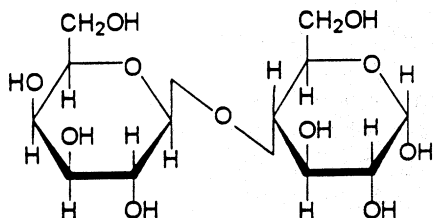**Figure 1.1** Structural formula of α-lactose.

α- and β-lactose. The structural formula for α-lactose (Figure 1.1) may be readily converted to that of the β-form by interchanging the OH and the H on the reducing group (C1 of the glucose moiety). The α- and β-configurations are distinguishable from one another since the β-form always has the hydroxyl group above the ring in Haworth structures. ^{13}C-NMR assignments have been studied (Pfeffer *et al.*, 1979, 1983).

1.3.1 Mutarotation

Regardless of the form of lactose when put into solution, the optical rotation will change with time as equilibrium between the α- and β-forms occurs. The change in rotation and the conversion of one form to the other in solution is called mutarotation. Mutarotation in solution may be followed by polarimetric measurements; this behaviour provides a useful method for determination of, for example, the amount of crystalline lactose present in spray-dried whey powders (Sharp and Doob, 1941; Pallansch, 1973).

Detailed discussions of the mutarotation of lactose are described elsewhere (Walstra and Jenness, 1984; Holsinger, 1987). The specific rotation of the α and β forms in water at 20°C is +89.4° and +35°, respectively. At equilibrium, $[\alpha]^{20}_D = +55.3°$ (anhydrous weight basis), equivalent to 37.3% α and 62.7% β; equilibrium rotation is the sum of the individual rotations of the α and β forms. The equilibrium ratio of β to α at 20°C, therefore, is 1.68 (62.7/37.3). The proportion of lactose in the α-form increases slightly as the temperature increases but the equilibrium value is not affected by pH. Mutarotation is a first-order reaction, the rate of which increases sharply with increasing temperature. Mutation rate is defined as:

$$K = k_1 + 10^{-4} \text{ s}^{-1} \text{ at } 20°C.$$

The temperature coefficient, Q_{10}, = 2.8 and the apparent activation energy is 75 kJ mol^{-1} (Walstra and Jenness, 1984). Mutarotation rate is also affected by pH, being minimal at about pH 5.0, and increasing rapidly at pH values <2 and >9 (Holsinger, 1987). The salts in milk increase

mutarotation by a factor of two compared with water (Walstra and Jenness, 1984). A high concentration of sugar, such as that found in sweetened condensed milk, significantly reduces the rate of mutarotation (Walstra and Jenness, 1984).

The specific rotation of lactose may also vary with the solvent. It is lower in alcoholic or acetone solutions but higher in glycerol than in aqueous solutions (Nickerson, 1974). The equilibrium ratio of α to β may also be altered by the nature of the solvent. As an example, when diluted with water, concentrated solutions in methanolic $CaCl_2$ show a high initial to final rotation, regardless of the form of lactose used to prepare the mixture (Domovs and Freund, 1960).

1.3.2 Solubility

Among sugars, the solubility of lactose is low. However, lactose solutions can become highly supersaturated before crystallization occurs. The α and β forms have distinctly different solubilities; mutarotation is also a factor in solubility. When an excess of α monohydrate is dispersed in water, a definite amount (about 7 g per 100 g, 15°C) dissolves; this initial solubility is the true solubility of the α form. Increasing solubility with time is due to mutarotation; as the α form is converted to β, the solution becomes unsaturated with respect to α and more α monohydrate dissolves. The process continues until a final equilibrium is reached and final solubility is established (about 17 g per 100 g, 15°C). β-Lactose, under similar conditions, shows considerably higher initial solubility in water (about 50 g per 100 g, 15°C). On mutarotation, more α-lactose forms than can be maintained in solution and crystallization occurs until equilibrium is re-established. Some solubilities of lactose are listed in Table 1.2.

Solutions of lactose can become highly supersaturated before crystal-

TABLE 1.2
Lactose solubility (g per 100 g water)

°C	α	Initial β	Final	Super solubility
0	5.0	45.1	11.9	25
15.0	7.1	–	16.9	38
25.0	8./6	–	21.6	50
39.0	(12.6)[a]	–	31.5	74
50.0	17.4	–	43.7	–
90.0	60.0	–	143.9	–
100.0	–	(94.7)	157.6	–

[a]Calculated values assuming $K = 1.5$ and solubility of one form is independent of the other (from Herrington, 1948, and Whittier, 1944).

lization occurs. Generally, the supersolubility at any temperature is equal to the saturation value at a temperature 30°C higher; the supersaturation values are readily reproducible. The ability of lactose to form supersaturated solutions and its relative insolubility are of considerable importance in the manufacture of a variety of dairy products, so the phenomenon of lactose crystallization has been studied widely (Walstra and Jenness, 1984; Holsinger, 1987). The concept of supersaturation was introduced by Ostwald in 1897 and extended to 'metastable' and 'labile' areas (Mullin, 1961). Solubility curves based on these concepts have been produced and are shown in Figure 1.2 (Hunziker, 1946). The metastable area occurs during the first stages of supersaturation caused by cooling a saturated solution or by continued evaporation beyond the saturation point. Since crystallization does not occur readily in this supersaturation range, seeding with lactose crystals is usually needed. Induced crystallization usually occurs at concentrations of about 1.6 times the final lactose solubility; the labile zone is found at concentrations >2.1 times the final lactose solubility (Walstra and Jenness, 1984).

1.3.3 Crystalline forms

The usual crystalline form obtained from cheese whey or water solutions concentrated to the saturation point at temperatures <93.5°C is the α-lactose monohydrate. The crystals may take a variety of shapes,
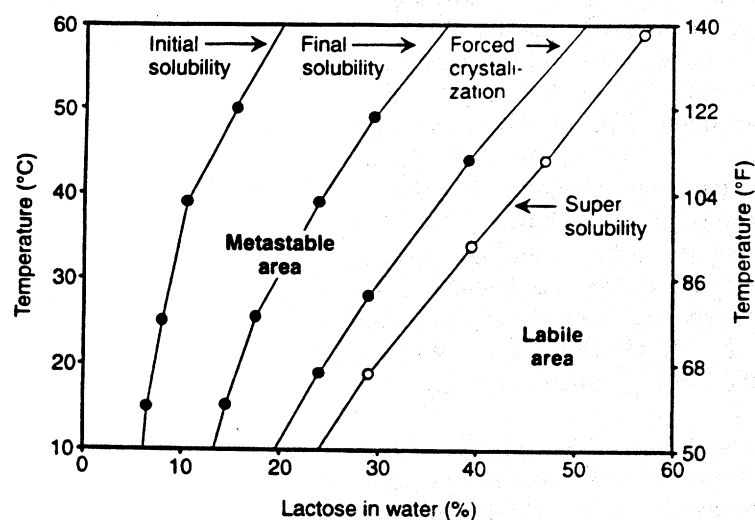
Figure 1.2 Lactose solubility curves (from Hunziger, 1946, with permission of the publisher).

depending on crystallization conditions. All the crystal habits of lactose found in dairy products are crystallographically equivalent to the tomahawk shape; a full description of the various crystal habits and factors affecting their formation and growth may be found in Holsinger (1987) and Walstra and Jenness (1984). The crystals are hard and gritty in the mouth; the texture is 'sandy' when the crystals exceed 16 μm in size. The influence of additives on crystal growth has been studied; in some cases, there is marked retardation (such as occurs in ice cream when gelatin or various marine and vegetable gums are added) and, in other cases, the additives (e.g. methanol, ethanol) cause accelerated growth on specific crystal faces. Other inhibitors of crystallization include milk components such as riboflavin, β-lactose and lactose monophosphate (Walstra and Jenness, 1984).

Crystal forms of anhydrous α-lactose may be produced when the water of crystallization is removed. When the α-hydrate is heated above 100°C under vacuum, a hygroscopic (unstable) form of anhydrous α-lactose occurs, which readily reforms the hydrate without dissolving when exposed to moisture (Holsinger, 1987). A stable anhydrous form (non-hygroscopic) may also be prepared by heating in air while holding the environment at a water vapour pressure between 6 and 80 cm mercury. The stable form differs from the unstable form in that it is denser, not very hygroscopic, must dissolve in water before forming the α-hydrate and is more soluble in water than either the α-hydrate or β-lactose (Holsinger, 1987). Other forms have also been described (Morrissey, 1985; Holsinger, 1987). Molecular complexes have been formed by shaking the α-hydrate with ten times its weight of methanol containing 1–5% of anhydrous HCl. Partial mutarotation of the α- to the β-form may occur and the complex precipitates with an α/β ratio of 5:3; this is not a mixture but a molecular compound containing $\alpha_5\beta_3$, as shown by X-ray diffraction and IR spectroscopy (Bushill *et al.*, 1969). A product with an α/β ratio of 3:2 is formed by NH_4OH in methanol (Olano *et al.*, 1977).

β-Lactose is formed when crystallization occurs above 93.5°C; the crystals are anhydrous. The common crystal form is an uneven-sided diamond when crystallized from water and curved needle-like prisms when crystallized from ethanol. The reported lattice constants are: $a = 10.81$ Å; $b = 13.34$ Å; $c = 4.84$ Å; $\beta = 91°15'$ (Buma and Wiegers, 1967). Some physical properties of the different crystalline forms are given in Table 1.3.

Amorphous lactose is formed when a solution is dried rapidly, such as in spray drying, or frozen. Although referred to as a glass, it may really be considered to be a concentrated solution that is quickly diluted upon adding water (Walstra and Jenness, 1984). The product is highly hygroscopic, readily sorbing water and crystallizing as α-monohydrate when the moisture content reaches about 8%. Very small crystals form under these

TABLE 1.3
Physical properties of crystalline forms of lactose[a]

	Melting point (°C)	Density (g cm⁻³)	Optical (rotation) ($[\alpha]^D_{20}$)
α-Hydrate	221		+01.1
	201.6	1.545	+89.8
	217	1.537	+89.8
α (unstable)	222.8	1.540	
α (stable)	216	1.540	
β-Lactose	235	1.587	+33.5
	252.2		+35
	229.5	1.590	+35
Complex α/β (4/1)	204	1.573	

[a]From Morrissey (1985), where original references may be found.

conditions, but if this occurs in dry milk, whey or spray-dried butteroil encapsulated in lactose, the powder particles will cement themselves together, forming a solid lump, as many manufacturers have learned to their sorrow!

1.4 LACTOSE MANUFACTURE

Crude or refined lactose may be prepared by crystallization either from cheese whey or from whey deproteinized by ultrafiltration or by heat coagulation. These crystallization processes are well established; although a variety of wheys and whey permeates may serve as starting material, sweet whey or ultrafiltrates are preferred (Woychik, 1982). For many years, only USP grade was produced but since lactose is now used in diverse products, a variety of grades are now available. Some typical compositional data for the various grades may be found in Table 1.4.

The crystallization process for producing lactose has three basic steps: (1) condensing the whey by multi-stage vacuum evaporation to 50–70% total solids, depending on the protein content of the whey; (2) crystallization (spontaneous or by seeding); (3) removal of the crystals by centrifugation. Since the yield and purity of the crystals are affected by the protein and mineral content of the starting material, it is best to begin with deproteinized, demineralized whey. The removal of minerals by ion exchange or by electrodialysis improves heat transfer in the evaporator by reducing deposit formation and permits concentration to 70–75% total solids; the product yield is increased by 10% since lactose crystallization is not interfered with by crystallization of salts (Caric, 1994). If the total

TABLE 1.4

Compositional data (%) for various lactose grades[a]

Component	Fermentation	Crude	Edible	USP[b]
Lactose	98.0	98..4	99.0	99.85
Non-hydrate moisture	0.35	0.3	0.5	0.1
Protein	1.0	0.8	0.1	0.01
Ash	0.45	0.40	0.2	0.03
Fat	0.2	0.1	0.1	0.00
Acidity as lactic acid	_[c]	_[c]	< 2	< 1

[a]From Morrissey (1985). Additional information may be found in Holsinger (1987).
[b]USP, US Pharmacopoeia grade.
[c]Not normally determined.

solids content of the condensed whey is too great, high viscosity makes crystal separation and washing almost impossible. During the centrifugation step, the crystals are sprayed with water to remove adhering liquor. The crystals may then be dried to produce crude lactose or redissolved, decolourized using activated carbon, filtered, concentrated and recrystallized or spray dried. Commerical practice applies numerous modifications of the basic steps described above; additional details may be found in available reviews (Nickerson 1974; Brinkman, 1976; Hobman, 1984; Holsinger, 1987; Caric, 1994).

Some improved procedures for the production of lactose have been reported (Harju et al., 1990; Hramtsov et al., 1990; Singh et al., 1991).

Hramtsov et al. (1990) produced a product of 98% total solids, 96% of which was lactose, by microfiltration, followed by pH adjustment and heat to remove protein; the refined microfiltrate was concentrated further by reverse osmosis, demineralized using ion exchange membranes, concentrated to 50% total solids and spray dried.

Harju et al. (1990) employed an ion exclusion and molecular sieve approach to treat the 'mother liquor' to improve the yield of lactose and simultaneously to produce a whey protein concentrate. They used a functionalized 'Merrified'[TM] resin (sulphonated polystyrene cross-linked with divinylbenzene) in sodium form and sieved to a particle size of 0.36 mm. A batch of clarified 'mother liquor' was pumped to the column, from which it was eluted with deionized water. By careful choice of conditions, it was possible to elute a whey protein fraction with a protein content >40% and a lactose fraction of purity between 75 and 95%, depending on where the cut was taken. The lactose fraction could then be returned to the crystallization vat in the manufacturing process. The process has been scaled up and a commercial operation has been started in Finland.

Singh *et al.* (1991) developed a one-step crystallization process for the production of edible and USP grade α-lactose monohydrate from ultrafiltered sweet cheese whey permeate at room temperature. This process is based on crystallization from ethanol (Leviton and Leighton, 1938). Edible lactose is produced from a clarified ultrafiltered permeate (30% total solids); constant stirring at pH 2.5–2.75 of an 80% ethanol solution yields 88% of the total lactose in 3 h. Addition of 1% acidic ethanol and further agitation for 10 min reduces trapped ash and protein. The slurry is centrifuged, and the crystals thus obtained are washed with cold water (10% v/v) and dried. USP grade lactose can be produced by demineralizing the concentrated permeate before treatment with ethanol: lactose monohydrate of 99.92% purity can be produced in one step by crystallization from 72.9% ethanol at pH 4–5 at ambient temperature.

The normal article of commerce is α-lactose monohydrate; there is only a limited demand for β-lactose, which is usually produced by crystallizing above 93.5°C (Holsinger, 1987).

1.5   Lactose in dairy products

Lactose and its crystallization are of critical importance in the manufacture and storage of a variety of dairy products. Crystallization principles and behaviour must be understood and applied in dairy processing if stable high-quality products are to be produced.

1.5.1   Condensed products

The control of lactose crystallization in sweetened condensed milk has been reviewed extensively (Hunziker, 1946; Webb, 1970; Hall and Hedrick, 1971; Morrissey, 1985), most recently by Caric (1994). There has been little research on this product in the United States in recent years, although a recent US patent (Little, 1991) describes a procedure for making sweetened condensed milk in a freeze-dried crystalline form that has a prolonged shelf-life. Caric (1994) also described several other approaches: in one case, sweetened condensed milk with hydrolysed lactose is reported to have the same sweetness as the conventionally processed product with the addition of sucrose. Sugar (sucrose) syrup may also be prehydrolysed by acid to prevent lactose crystallization. The application of ultrafiltration to produce sweetened condensed milk has also been studied; the product has only about 11% lactose compared with 42% in the conventional product. Advantages of this approach include a lower viscosity, no formation of large crystals and no colour changes due to the Maillard reaction.

Frozen concentrated milks have always been regarded as an attractive

way of preserving milk with minimal flavour change. Freezing of milk is almost always accompanied by destabilization of the casein, the cause of which is the high concentration of milk salts in the unfrozen portion of the milk which occurs after lactose crystallization and freezing of the water in which the lactose had been previously dissolved. Calcium phosphate also precipitates on freezing, causing a decrease in pH. The combination of these two factors alters the stability of the casein micelles (Morrissey, 1985). Protein destabilization may be retarded by treatments that destroy the availability of lactose nuclei for the initiation of crystallization or by reducing the rate of crystallization. An example of such an application is the partial enzymatic hydrolysis of the lactose before concentration (3:1), followed by a post-pasteurization heat treatment at 71°C for 30 min after canning; samples showed only a moderate increase in viscosity after 9 months of frozen storage (Holsinger, 1978). Using this method, Guy (1982) successfully increased the physical stability of frozen goats' milk concentrates (4:1, 48% total solids).

Conditions found in ice cream and other frozen desserts also lead to lactose crystallization and destabilization of the casein. A defect called 'sandiness', an objectionable gritty texture, is the result of lactose crystallization. However, this defect has virtually disappeared with the development and widespread use of various marine and vegetable gums and other stabilizers, which function by inhibiting the formation of lactose crystal nuclei (Nickerson, 1962).

1.5.2 Dried products

The major constituent of most dried dairy-based food powders is lactose. There is about 37% lactose in dry whole milk, 50% in non-fat dry milk, 50% in buttermilk powder and 70% in whey powder; whey permeates contain even higher levels. If lactose is the encapsulating agent, spray-dried butteroil contains a significant amount (Onwulata et al., 1994).

(a) Milk powders

Lactose in milk powders is either amorphous or partially crystalline, depending on the processing conditions. Because the lactose glass is highly hygroscopic, dried milk particles readily absorb moisture from the air; the lactose molecules acquire sufficient mobility and space to orient themselves into a crystal lattice. When this happens, the powder particles cake and clump into a hard mass (Troy and Sharp, 1930).

The moisture sorption behaviour of various milk powders has been reported (Berlin et al., 1968a,b, 1973). A detailed discussion of the moisture sorption properties of lactose systems may be found in Morrissey (1985). The sharp discontinuities observed in the water sorption isotherms of lactose-containing dairy products at about A_w of 0.5 is

attributed to the transformation of lactose from the metastable amorphous state to the crystalline form. Berlin et al. (1968a,b), who compared moisture sorption isotherms for milk protein, lactose and salts (Jenness–Koops synthetic milk salt system), demonstrated that there is a progressive order of moisture sorption by the milk components as A_w increases. First, the casein sorbs water and swells; at an A_w of 0.2 to 0.6, the whey proteins bind moisture, followed by the lactose which crystallizes; finally, as A_w increases beyond 0.6, the salts rapidly sorb moisture. Careful moisture control in high-lactose products is essential to maintain quality; dry milk should be kept below 6% moisture ($A_w = 0.4$), to prevent lactose crystallization.

Because milk powder can be difficult to reconstitute, especially in cold water, partly because of the low solubility of lactose, most milk powder for retail sale is 'instantized' for the purpose of improving the rate of reconstitution. The original patent for this procedure was granted to Peebles (1956). There are two basic types of instantizing: re-wetting, where the dried powder particles are re-wetted with steam and re-dried to form clusters of powder particles by agglomeration, and a 'straight-through' process, where agglomeration is carried out during drying, immediately after powder particles have been formed (Caric, 1994). Agglomeration, or sticking together of the powder particles, involves some crystallization of amorphous lactose (Morrissey, 1985). SEM studies clearly show the presence of microcrystalline lactose in agglomerated powder particles (Caric and Kalab, 1987). The use of added lactose as an aid in instantizing is an acceptable practice in the United States; proposed revisions in the non-fat dry milk standards would restrict the use of lactose to a maximum of 2% of the total weight of the non-fat dry milk (Anonymous, 1995).

(b) Dry whey and whey permeate

Lactose, comprising about 70% of the total solids of cheese whey, obviously plays a major role in determining the properties of dry whey and whey products. Since whey may be difficult to dry, modifications of the roller and spray processes used to dry milk are necessary. These usually consist of procedures to cope with the highly sticky, hygroscopic lactose glass formed during dehydration; therefore, the target in whey powder production is to convert as much of the lactose as possible into the crystalline form. To accomplish this, the product is held at some stage of processing, in the presence of sufficient moisture to ensure crystallization of the α-hydrate.

Caric (1994) described four possible procedures for spray drying of whey. Three of the procedures lead to free-flowing powders, with the bulk of the lactose in the crystalline form. A feature of all three processes is the concentration of whey to 45–60% total solids, pre-crystallizing for 4–24 h and spray drying. Since crystallization of lactose in high solids whey

is accompanied by a rapid increase in viscosity, many whey drying operations carry out the major portion of lactose crystallization after spray drying. In two of the processes described by Caric (1994), drying conditions are controlled so that sufficient moisture remains in the damp powder to permit lactose crystallization to continue; the residual moisture is then removed and the powder cooled in a secondary drying system, a vibrating fluidized bed; in one case, further crystallization continues on the crystallization belt conveyer leading to the vibrating fluid bed. Powder manufactured in this manner consists of large agglomerates of free-flowing powder with 85–95% of the lactose in the crystalline form.

Foam-spray drying was developed for the purpose of drying high-solids acid whey (Hanrahan and Webb, 1961a,b), which, in addition to its high lactose content, contains significant levels of lactic acid. By injecting compressed air into the whey just prior to the spray nozzle, a foam structure resulted that dries rapidly, forming a free-flowing powder. Hygroscopicity problems were overcome by precrystallizing the lactose in the concentrate before drying (Tamsma et al., 1972). Sticking of whey solids to the hot metal surfaces of the drying chamber can be a problem; the temperature at which sticking occurs is dependent on lactic acid content, amorphous lactose and moisture (Pallansch, 1973). If sufficient moisture is present to allow lactose crystallization to proceed in the drying chamber, a point will be reached at which the powder no longer adheres to the equipment. Fluidized bed drying is ideal for drying acid or cottage cheese whey.

Spray drying of the permeate from ultrafiltration operations was investigated by Hargrove et al. (1976). Under the experimental conditions used, concentrates with 40–50% total solids dried readily but concentrates above 50% total solids failed to dry. Powders with a high level of crystalline lactose were prepared by concentrating the permeate to 60% total solids, holding with agitation at 40 C for 1–2 h, adding water to 50% total solids and spray drying. These researchers found a direct correlation between lactic acid content and powder hygroscopicity but no correlation between crystalline lactose content and hygroscopicity.

(c) Butter powder
Spray drying anhydrous butteroil with functional encapsulants such as whey proteins or disaccharides and other carbohydrates to form free-flowing powders can enhance stability by forming fat-containing microcapsules during the drying process (Young et al., 1993; Onwulata et al., 1994). Lactose and other disaccharides have been used successfully to encapsulate 40 or 60% milkfat by spray drying; the lactose content of the finished powder ranged from about 31 to 51%, depending on the fat content. In a comparative study, moisture sorption isotherms of spray dried milkfat encapsulated in lactose, maltose or sucrose, show patterns typical of an amorphous powder that desorbs moisture upon crystal-
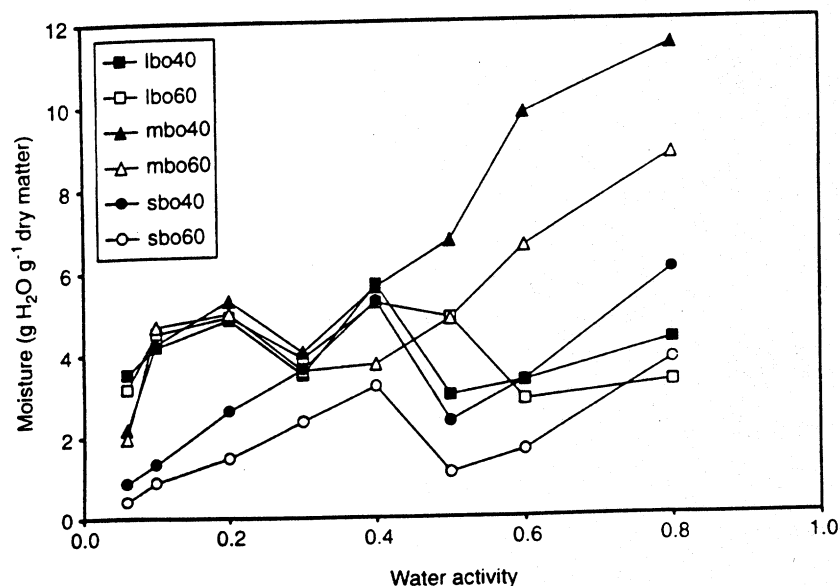
Figure 1.3 Moisture sorption isotherms of anhydrous butteroil encapsulated in lactose, maltose or sucrose at the 40% or 60% level. lbo40 = lactose, 40% butteroil; lbo60 = lactose, 60% butteroil; mbo40 = maltose, 40% butteroil; mbo60 = maltose, 60% butteroil; sbo40 = sucrose, 40% butteroil; sbo60 = sucrose, 60% butteroil.

lization (Figure 1.3). The powders with the higher levels of encapsulant absorbed more total moisture. Powders with lactose or maltose as the encapsulant desorbed water upon crystallization at an A_w of around 0.2, whereas sucrose-containing powders did not show a break in the curve until an A_w of around 0.4. This means that microcapsules formed with lactose during spray drying will fuse and partially dissolve, releasing the milkfat, as a result of moisture uptake and subsequent crystallization. Special packaging of these powders is necessary to prevent moisture uptake during long-term storage.

1.6 CHEMICAL REACTIONS

Since lactose is like a number of similar carbohydrates, it reacts according to the general rules of carbohydrate chemistry. The reactions may involve (1) the glycosidic linkage between the constituent monosaccharides, (2) the reducing group of the glucose moiety, (3) free hydroxyl groups, and (4) carbon–carbon bonds. Discussions of the chemical properties of lactose are available (Clamp et al., 1961; Doner and Hicks, 1982; Thelwall, 1985).
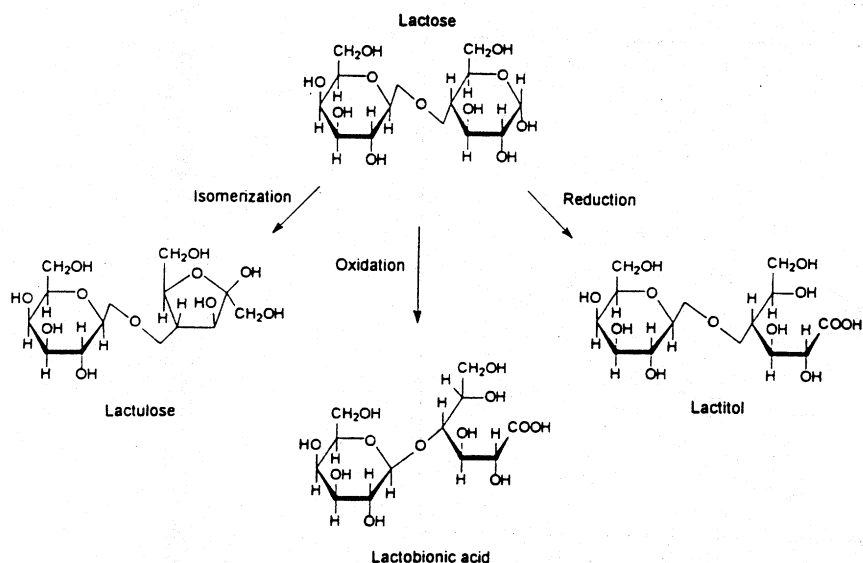
Figure 1.4 Structural formula for α-lactose and its conversion into lactulose, lactitol or lactobionic acid.

The three major derivatives of lactose, in which the β 1–4 linkage remains intact, are lactulose, formed by isomerization, lactitol, produced by reduction, and lactobionic acid, produced by oxidation (Figure 1.4). Other derivatives include gluconic and other acids, lactosylurea, N-substituted amino sugars, and polymers; other reactions include esterification and acetylation (Holsinger, 1987). Enzymatic hydrolysis, however, has had the greatest impact on the dairy industry, and has led to the commercial availability of lactose-modified milk products in many countries around the world.

1.6.1 Lactulose

Lactulose (4-*O*-D-fructofuranose) is an isomer of lactose, formed by molecular rearrangement, frequently under alkaline conditions, where the terminal aldose residue of lactose is converted to a ketose. Doner and Hicks (1982) and Zadow (1984) have described the preparation and properties of lactulose in detail. Its formation and degradation in heated milk have been described (Andrews, 1989; Berg and van Boekel, 1994). World consumption of lactulose (50% solution) is estimated to be about 20 000 tonnes per year (Tamura *et al.*, 1993).

A new crystalline form of lactulose, a trihydrate, has been reported recently (Tamura *et al.*, 1993). It contains 86.4% lactulose and 13.6% water, has a melting point of 68°C, a solubility of about 53% in water at 15°C, and a heat of solution of 34 kJ mol^{-1} at 5%, w/w. It crystallizes readily from water and is stable at 30°C and 81% RH. It loses its water of crystallization at temperatures above 35°C so it must be stored at or below ambient temperature.

Lactulose has important uses in the pharmaceutical industry. The major uses are in treatment of portal systemic encephalopathy and chronic constipation (Doner and Hicks, 1982; Tamura *et al.*, 1993). Portal systemic encephalopathy is manifested when ammonia, produced in the intestine, is not removed from the blood by the liver; lactulose regulates intestinal activity, stimulates the growth of *Bifidobacterium*, and suppresses ammonia production by intestinal bacteria and lowers ammonia absorption. Lactulose may also aid in controlling *Salmonella* colonization in the large intestine (Hoffmann, 1975).

Although the preparation of lactulose with $Ca(OH)_2$ had long been known, it was not until the report of Hicks and Parrish (1980) that lactulose could be produced in more than 90% yield; they treated lactose with boric acid in aqueous solution made basic by the presence of tertiary amines. Hicks *et al.* (1984) also demonstrated that yields of lactulose exceeding 80% could be produced from sweet whey ultrafiltrate; they described five purification processes that allowed the crystallization of pure non-hygroscopic lactulose from syrups. More recent research (Kozempel and Kurantz, 1994a,b) has led to the development of a continuous reactor system to produce lactulose based on the use of the borate ion and NaOH to isomerize lactose. The goal of the research was to develop a continuous pilot plant process and gather the data needed for scale-up to a commercially feasible process.

Tamura *et al.* (1993) describe the purification of lactulose industrially by ion exchange chromatography, using a sodium-type strong acid ion exchange resin column. Lactose, lactulose and galactose are eluted in order with water. These authors also described a pilot plant for the continuous industrial separation of lactulose from other sugars by chromatography, using a computer-controlled 'simulated moving bed'. Lactulose solution and water are injected and pure lactose and other sugars are obtained. The Morinaga Milk Industry Co. Ltd of Japan produces lactulose syrup (MLS-50), a highly purified lactulose syrup (MLS-95), lactulose powder (MLP-40), lactulose crystals (trihydrate, MLC-H), and lactulose crystals (anhydrous, MLC-A). These products are used in a variety of food and pharmaceutical products in Japan.

Lactulose is applied as a 'nutraceutical' in some sectors of the food industry, especially infant formulae. Lactulose was approved in 1992 in Japan as a special food for maintenance of health and protection against

enteric infections (Tamura *et al.*, 1993). Morinaga produces an infant formula that contains 0.5% lactulose, lactoferrin and peptides; infants fed on this product have fecal microflora very close to those of infants fed on breast milk. Morinaga also produces a follow-up formula containing 0.4% lactulose. Another product, Sawayaka Milk, contains 4% lactulose and more than 1×10^8 viable *Bifidobacteria* per g; it is intended to regulate intestinal function.

1.6.2 Lactitol

Lactitol (4-*O*-D-galactopyranosyl-D-sorbitol) is a disaccharide sugar alcohol made industrially from lactose by catalytic hydrogenation with Raney nickel; lactitol is produced in nearly quantitative yield (van Velthuijsen, 1979). Food applications include frozen desserts, confectionery and baked products.

Lactitol is readily crystallized as either the mono- or dihydrate, depending on temperature and concentration (Blankers, 1995). It is not a reducing sugar and is very stable under acid and alkaline conditions and at high temperature. Lactitol is one of the least hygroscopic polyols (Blankers, 1995); the monohydrate is less hygroscopic than sorbitol and xylitol but more hygroscopic than mannitol.

At room temperature, lactitol attains the solubility of sucrose, so many of its applications are as a substituent for sucrose. Its molecular weight is equivalent to that of sucrose so its effects on freezing point depression and A_w are similar. Its relative sweetness is about 35% of that of sucrose (van Velthuijsen, 1979); for example, an 11.4% solution has sweetness equal to that of a 4% sucrose solution. Its cooling effect (because of a negative heat of solution) is mild and not as pronounced as that of sorbitol or xylitol (Blankers, 1995).

Lactitol can be tolerated by diabetics. Clinical trials have shown that its consumption does not increase blood glucose or insulin levels (Blankers, 1995). Apparently, lactitol is not absorbed or hydrolysed in the small intestine; instead, it is fermented in the large intestine to biomass and short-chain fatty acids. Lactitol lowers the colonic pH in a manner similar to lactulose (Patil *et al.*, 1987); it has also been shown to be effective in the treatment of hepatic encephalopathy and may also affect the metabolism of bile acids (Harju, 1993). It has a reduced caloric value of 2 kcal g^{-1}, i.e. 50% of that of sucrose.

Lactitol is non-cariogenic. Early studies showed that lactitol was not readily fermented by *Streptococcus mutans* and other oral bacteria so its consumption does not lead to acids that demineralize tooth enamel; formation of plaque is much less when compared with sucrose (van Velthuijsen, 1979). The non-cariogenic properties have been confirmed in clinical trials (Grenby, 1989).

The safety of lactitol has been confirmed in many animal and human studies. It has been approved for food use in several European countries, Japan, Australia and Canada (Blankers, 1995). The joint FAO/WHO Committee on Food Additives has recommended a 'not specified' accepted daily intake (Blankers, 1995). PURAC biochim b.v. filed a self-affirmation petition for the GRAS status of lactitol with the US Food and Drug Administration in September 1993, which has been accepted (US FDA, 1993; Blankers, 1995). The scope of the GRAS petition includes chewing gum, hard and soft candy and frozen dairy desserts.

1.6.3 Lactobionic acid

Lactobionic acid (4-O-β-galactopyranosyl-D-gluconic acid) is not normally found in nature but may be readily produced from lactose by specific oxidation of the free aldehyde group (Harju, 1993). High yields of lacto-bionic acid may be obtained catalytically, electrochemically, chemically, enzymatically or by fermentation (Roelfsema and Kuster, 1988; Hendriks et al., 1990). If lactobionic acid is to be manufactured on a large scale, catalytic oxidation with palladium or platinum, using bismuth as a promoter, appears to be the most promising (Hendriks et al., 1990; Fuertes and Fleche, 1991).

Lactobionic acid can be used as a chelating agent for heavy metals, such as iron, under conditions where EDTA is not effective. However, gluconic acid proved to be about twice as effective for this purpose (Scholnick and Pfeffer, 1989). Calcium, cupric and ferric salts of lacto-bionic acid have been prepared; the salt is a good source of calcium in pharmaceutical preparations. Calcium lactobionate has been defined as a food additive for use as a firming agent in dry pudding mixes (Code of Federal Regulations, 1994).

Solvay Deutschland GmbH, Hannover, Germany, produces lactobionic acid; both sodium and potassium lactobionate are available, either spray dried or as liquid concentrates with 60% total solids. The company proposes an application of potassium lactobionate in alkaline liquid or powder detergent formations containing either perchlorate bleach or added borate and has demonstrated its effectiveness in washing trials with cotton and cotton/polyester test materials (Solvay Deutschland GmbH, 1994.

In addition to electrolytic oxidation, lactobionic-δ-lactone, which readily hydrolyses to the acid, may be formed by the direct enzymatic conversion of lactose by lactose dehydrogenase produced by *Pseudomonas graveolens* (Wright and Rand, 1973; Qadeer et al., 1974). The lactone may also be reacted with amines to form stable amides (Scholnick and Pfeffer, 1980); no antimicrobial activity or other special use for these derivatives was identified. More recently, lactobionate has proven to be the most

important component in UW solution for liver preservation for organ transplants (Sumimoto and Kamada, 1990); it is used commercially in the Viaspan™ solution of the DuPont Co., Wilmington, DE. Lactobionic acid apparently suppresses cell swelling during hypothermic storage; its functionality is not completely understood although the ability of lactobionic acid to chelate iron may be important.

1.6.4 Other derivatives

(a) Gluconic and other acids

Zadow (1984) reported a process to convert lactose to gluconic acid and galactose under acidic conditions and treatment with bromine; galactose is recovered from the product concentrate by crystallization. D-Glucaric (saccharic) and D-galactaric (mucic) acids are formed by treatment of lactose with nitric acid; if the acid is concentrated or hot, further oxidation to tartaric, oxalic and carbonic acid may occur. In alkaline solution with potassium permanganate, complete oxidation to carbon dioxide and water can be carried out; microbial action may also degrade lactose to carbon dioxide and water.

(b) Lactosylurea

Cerbulis *et al.* (1978) studied the chemical characteristics of the reaction of lactose with urea, demonstrating that maximum yields (40%) could be obtained at an initial pH of 2.0. Above pH 4.0, lactulose was the principal secondary product formed; the yield of lactulose increased to 15% at pH 7.0.

(c) Other reactions

The reductive amination of lactose with alkylamines and sodium cyanoborohydride under weakly acid conditions in boiling methanol has been carried out by Hoagland *et al.* (1979). Polyurethane foams have been successfully formed by reaction with dimethyl sulphoxide (Hustad *et al.*, 1970). Thelwall (1985) discussed other chemical reactions of lactose in detail, for example, regioselective esterification and acetylation reactions. The partially protected derivatives formed were of value in further modification of lactose and aided in the synthesis of higher oligosaccharides.

1.6.5 Heat

The reactions of lactose in heated milk have been studied intensively: in the 1950s and later, efforts were centred around the Maillard reaction (O'Brien and Morrissey, 1989), but, more recently, with the development of UHT processing, attention has been paid to the isomerization of

lactose to lactulose by heat (Andrews, 1989; Olano and Martinez-Castro, 1989). Most recently, Berg and van Boekel (1994) have established two reaction pathways when they studied milks heated between 110 and 150°C: (1) lactose isomerization to lactulose, and (2) the Maillard reaction. Under their experimental conditions, 80% of the lactose reacted by the isomerization route while only 20% reacted *via* the Maillard reaction.

(a) Maillard reaction

Milk is the only major naturally occurring proteinaceous food with a high content of a reducing sugar. Patton (1955) published an extensive review of browning and other associated changes in milk; O'Brien and Morrissey (1989) summarized the Maillard reaction in milk products.

Mild heat treatment of milk results in changes in colour, flavour, functionality and nutritive value. The overall reaction may be divided into three stages: (1) condensation of the reducing group of lactose with the free epsilon amino group of lysine to form a Schiff's base, followed by the formation of an N-substituted glycosylamine; (2) a spontaneous and irreversible rearrangement of the glycosylamine *via* an Amadori rearrangement to form a 1-amino-1-deoxy-2-ketose; (3) reaction of the relatively stable Amadori compounds principally by two routes, the choice of pathway being affected by pH. At low pH, after enolization and further degradation, furfurals or hydroxymethylfurfurals are formed. At high pH, after enolization, elimination of the allyl amine residue and formation of a 1-methyl-2,3-dicarbonyl intermediate, further decomposition forms a variety of compounds such as pyruvaldehyde, diacetyl, hydroxydiacetyl, acetylfuran, pyrones and maltol. A third pathway involves the Strecker degradation, the interaction of amino acids and dicarbonyl compounds (dehydroreductones, dehydration or fission products) produced from the breakdown of the Amadori compounds from pathways 1 and 2, or from fission products formed at high temperatures (O'Brien and Morrissey, 1989). Berg and van Boekel (1994) suggest that degradation of lactose, or formation of lactulose and galactose, cannot be described by a simple first- or second-order reaction since the reactions occur simultaneously and the reaction products can be formed by several pathways.

Browning has a detrimental effect on the nutritive value of milk products because the reaction of the sugar with the essential amino acid lysine becomes irreversible and biologically unavailable. Losses in lysine are 1–2% for pasteurization, 1–4% for UHT sterilization and 5% for brief boiling (Renner, 1983). About 20% of the available lysine is lost in the manufacture of evaporated milk (Renner, 1983) while losses in dried products depend on the manufacturing procedure. For example, lysine losses are 0–3% for spray dried milk powder, 20–75% for roller dried milk powder and 55% for spray dried lactase-treated milk powder

(Morrissey, 1985). Dried whey products containing lactose also undergo browning; Saltmarch *et al.* (1981) showed that browning was greatest in whey powders stored at $A_w = 0.44$, the point of discontinuity on the water sorption isotherm where amorphous lactose crystallized, releasing water that mobilized reactants for the browning reaction.

Erbersdobler and Dehn-Müller (1989) studied the formation of early Maillard products during the UHT treatment of milk. Using the 'furosine' method as an indicator of the formation of Amadori compounds (Erbersdobler, 1986), they showed that heating milk under UHT conditions forms lactuloselysine, the main Amadori compound resulting from the early stages of the Maillard reaction. They concluded that lysine losses were 1.2–2.4% in indirectly heat treated UHT milks, and lower in directly heated milks; the damage to lysine was unlikely to be of nutritional significance.

Although the browning reaction can be inhibited in milk products by such compounds as active sulphydryls, sodium bisulphite, sulphur dioxide or formaldehyde, in practice, browning is controlled by limiting heat treatment, moisture content and time and temperature of storage (Holsinger, 1987).

(b) Isomerization

Lactulose is found in two forms in heated milk: free and covalently bound to the free amino groups of the milk proteins as ε-N-deoxyl-actulosyl-L-lysine. Interest in lactulose formation in heated milks was stimulated because of possibilities for defining the severity of heat treatment given to the milk (Burton, 1984). Andrews (1984) clearly demonstrated that pasteurized, UHT-sterilized and retort-sterilized (in-can) milks could be distinguished from one another on the basis of their lactulose content; in addition, directly and indirectly processed UHT milks could be differentiated. Andrews (1989) has reported a number of compositional and technological factors that could affect the use of lactulose as a means of distinguishing UHT and sterilized milks. In summary, the lactulose concentration in UHT and sterilized milks varies on storage; it is both formed and degraded during storage, but formation is more temperature-dependent than degradation; visible light does not affect concentration during storage; as hydroxyl ion concentration increases, the rate of lactulose formation increases; lactulose formation is a first-order reaction with respect to lactose and obeys Arrhenius kinetics, with an activation energy of 122 ± 14 kJ mol^{-1}; dissolved oxygen and protein concentration do not affect lactulose formation; phosphate and citrate accelerate lactulose formation, citrate being more effective, while calcium suppresses formation, presumably by complexing with phosphate and citrate; dilution of the milk with water reduces lactulose formation proportionally; deposits on the walls of the heat exchanger affect formation only by their effect on the heat transfer characteristics.

Although a wide variety of methods have been used to determine the lactulose concentration in heated milks, enzymatic procedures were first evaluated for their accuracy and reproducibility (Andrews, 1989). More recently, however, an HPLC reference method and GC methods have been evaluated by the International Dairy Federation and a standard developed (IDF, 1991). According to Andrews (1984), the lactulose content for identification purposes is as follows: raw or pasteurized, 0 mg per 100 ml; UHT, 5–71.5 mg per 100 ml; sterilized, >71.5 mg per 100 ml. Direct UHT heating gives milks with lactulose contents of <9 mg per 100 ml whereas indirect UHT-heated milks have lactulose contents of >19 mg per 100 ml.

1.6.6 Hydrolysis

Lactose may be hydrolysed either enzymatically or by dilute solutions of strong acids. The initial products of acid hydrolysis are the component monosaccharides, glucose and galactose, in equal proportions. However, side reactions may occur and oligosaccharides may also form in concentrated solutions by reversion. Lactose hydrolysis has been reviewed recently (IDF, 1993).

(a) Acid hydrolysis
Compared with non-reducing disaccharides, lactose is relatively resistant to acid hydrolysis. Organic acids, such as citric acid, cannot hydrolyse lactose under conditions where they readily hydrolyse sucrose. The rate of hydrolysis of lactose varies with time, temperature and concentration. For example, a 33.6% solution of lactose can be 82% hydrolysed by HCl in 36 min at 130°C; a 29% solution can be 80% hydrolysed in 59 min at the same temperature (Ramsdell and Webb, 1945). Lin and Nickerson (1977) and Coughlin and Nickerson (1975), among others, hydrolysed 90% of the lactose at 60°C (5–40% solutions) with 1–3 N H_2SO_4 or HCl for reaction times up to 36 h. This process could not be adapted to whey concentrate because degradative side reactions produced high levels of off-colour and off-flavour. Demineralization and decolourization, by ion exchange and filtration through activated carbon, improve the quality of the hydrolysed syrups formed, but add to production costs (Chiu and Kosikowski, 1985).

Sulphonic acid-type ion exchange resins were also effective in hydrolysing lactose and had the added advantages of continuous operation, short reaction times and no contamination with mineral acids (Mulherin et al., 1979). The high temperatures (90 to 98°C) and low pH eliminated microbial growth. de Boer and Robbertsen (1981) used ultrafiltration permeate concentrated to 10% total solids for this purpose. The pH of the permeate was lowered to 1.2 by the strong acid cation exchange resin

used, followed by heat treatment in a heat exchanger at 150°C for 3 min; approximately 80% of the lactose was hydrolysed by this process.

(b) Enzymatic hydrolysis

The literature on the hydrolysis of lactose with β-galactosidase (lactase) enzymes is voluminous; recent reviews include Zadow (1984), Holsinger and Kligerman (1991) and IDF (1993).

The three major approaches to enzymatic hydrolysis are: (1) 'batch' or 'single-use' systems; (2) membrane-based lactase recovery systems to retain the enzyme for reuse; (3) immobilized systems where the enzyme is physically or chemically bound to a solid matrix

β-Galactosidase is produced by a variety of plant, animal and microbial cells. Commercially available enzymes are derived from yeasts such as *Kluyveromyces lactis* and *Kluyveromyces fragilis* and fungi such as *Aspergillus oryzae* and *Aspergillus niger*. The *K. lactis* enzyme has a pH optimum close to that of milk and exhibits considerable activity at 4–6°C, making it suitable for treatment of milk before consumption. *A. oryzae* β-galactosidase has a pH optimum of 5.0 and an optimum temperature of 50–55°C, making it functional in the acid environment of the stomach. *K. fragilis* and *A. niger* lactases, with pH optima of 4–4.5, good pH stability (pH 3.0–7.0) and temperature optima of 55°C, are suitable for hydrolysis of lactose in acid whey (Holsinger and Kligerman, 1991).

A batch processing system is the simplest means of producing lactose-modified milk but suffers from the disadvantage that a considerable amount of enzyme is needed. The concept of the batch system led to the first commercial development of lactose-reduced milk in the United States in the mid-1980s by the Sugar-Lo Company, the progenitor of LactAid Inc., Pleasantville, NJ (Holsinger and Kligerman, 1991). Lactose-reduced milk ($\geqslant$90% hydrolysis) was introduced in Ontario under the Lacteeze™ label in 1987 and is now sold across Canada (Modler *et al.*, 1993).

The development of a sterile filtered liquid form of the enzyme not only made it possible to produce a dairy-modified lactose-reduced milk but could also be used to introduce a shelf-stable hydrolysed-lactose UHT processed milk, provided that the enzyme was introduced after heat treatment into the pack before filling (Holsinger and Kligerman, 1991). The liquid form is also packaged for retail sale for use in the home. Tablet lines are also available for both consumers and health professionals; they contain lactase from *A. oryzae* and are designed to be taken with a meal. Palumbo *et al.* (1995) took advantage of this in their development of a beverage powder for field rations; the *A. oryzae* enzyme retained 93% of its activity after 6 months of storage at 45°C when it was dry blended with spray dried milk powder containing 2% fat.

Zadow (1984) reported on membrane reactor systems in which the enzyme is recovered by ultrafiltration after hydrolysis is complete.

Although pilot testing was done, problems with membrane fouling and microbial contamination have restricted the commercial adoption of this technology (Geilman, 1993).

Lactose hydrolysis with immobilized systems appears to be the method of choice when continuous production of hydrolysed syrups on a large scale is desired. Immobilization is done in several ways: absorbed on the surface, entrapped in beads or fibres or covalently bonded to a fixed medium (Geilman, 1933). Commercial processes have been developed, the best known of which are those of the Corning Glass Co. of the United States and the Valio Dairy of Finland. Semi-industrial plants were built to produce hydrolysed lactose syrups. Unfortunately, however, economic incentives to produce hydrolysed lactose syrups have disappeared because of depressed world sucrose prices, competition from high-fructose products, a shortfall of lactose in 1991, resulting in price increases, new uses for lactose in confectionery products and difficulties in drying the hydrolysed syrups (Modler, 1993).

Other approaches to deliver β-galactosidase to the substrate have been described. Jelen (1993) proposed that the cost effectiveness of hydrolysing lactose with soluble enzymes (batch operation) can be improved by using sonicated dairy cultures, especially high-lactase-producing strains such as *Lactobacillus delbrueckii* subsp. *bulgaricus*.

Somkuti and Steinberg (1990, 1991, 1993, 1994), in their studies of non-growing lactose transport systems of defective mutant cultures of *Streptococcus thermophilus*, have demonstrated that permeabilization with organic solvents or detergents allows the free passage of lactose to the cell interior where the β-galactosidase is still fully retained. The permeabilized cells may be viewed as enzyme 'microcarriers' in which the β-galactosidase remains in a naturally 'immobilized' state. They suggest that under industrial conditions, lactose hydrolysis in milk could be carried out at 50–55°C for up to 6 h before pasteurization or sterilization by adding 10^8 CFU per ml permeabilized and non-growing *S. thermophilus*. The extent of hydrolysis is regulated by controlling the treatment time, which could produce up to 90% hydrolysis of the lactose present in milk.

β-Galactosidases are also able to catalyse transferase reactions involving both lactose and its hydrolysis products, particularly galactose, to form a family of di- to hexasaccharides, termed galactooligosaccharides (Smart, 1993).

The reaction mechanism involved is transgalactosylation where the enzyme transfers the galactose moiety of a β-galactoside such as lactose, or an acceptor containing a hydroxyl group.

The source of the lactase dictates the number and type of oligosaccharides formed. The enzymes most studied have been those of commercial importance, i.e. those derived from the yeasts, *K. lactis* and *K. fragilis* or from the fungi, *A. oryzae* and *A. niger*. Each enzyme produces its own

distinct set of oligosaccharides; variation may be considerable. For example, Toba *et al.* (1985) identified 20 oligosaccharides produced on treatment with *A. oryzae* lactase. The galactooligosaccharides are invariably hydrolysed further by the lactase and appear only transiently in the reaction mixture; in concentrated lactose solutions, the transferase reaction products may reach concentrations in excess of those of the principal hydrolysis products, glucose and galactose (Smart, 1993).

These reactions are of considerable scientific interest because of their potential biological impact. The oligosaccharides formed during lactose hydrolysis have been seen as unwanted byproducts because of their poor digestibility, low sweetness and poor solubility. In contrast, the Yakult Honsha Co., Tokyo, Japan, has identified a product development opportunity involving the use of 'transgalactosyl oligosaccharides' in fermented milk drinks as specific growth factors for bifidobacteria. They are viewed as acting in the same manner as the compounds in human milk are believed to act in promoting growth of the *Bifidobacteria* in the human intestine. Yakult has developed a facility to produce 6500 tonnes of this preparation per year and add the preparation to their fermented milk drinks (Smart, 1993). The appropriate agencies in Japan have recognized oligosaccharides as physiologically functional food ingredients.

Smart (1993) also mentioned further uses for β-galactosidases: to develop heterologous oligosaccharides using mixed substrates and possibly more than one transferase to produce new and novel compounds. As an example, β-galactosidases have been used to form branched cyclodextrins with different functionality using lactose as a donor substrate (Kitahata *et al.*, 1992). Oligosaccharides may also be formed from chemical derivatives of lactose, such as lactitol, to form novel compounds (Yanahira *et al.*, 1992).

1.7 PHYSIOLOGICAL ASPECTS OF LACTOSE IN NUTRITION

Dairy products are important sources of many nutrients in the diet, including calcium, phosphorus, potassium, high quality protein and riboflavin. It has been estimated that up to 70% (28% of United States adults) of the world population have low levels of lactase activity in the intestine, and, as a result, a genetically controlled inability to digest lactose. Maldigestion of lactose can lead to a variety of unpleasant gastrointestinal symptoms which can prevent consumption of milk and its products. Even though most problems with lactose digestion are attributed to the lactose molecule, other problems may arise from galactose, liberated on hydrolysis. A large literature has developed as a result (Paige and Bayless, 1981; Delmont, 1983; Renner, 1983; Schaafsma, 1990; de Vrese, 1993; Miller *et al.*, 1994).

The significance of this subject is obvious because of its implications for the suitability of milk as a weaning food for populations where there is a high incidence of lactase deficiency, and the increased risk of disease from reduced calcium intake. The American Academy of Pediatrics stated the following in 1978 and again in 1990: 'On the basis of present evidence, it would be inappropriate to discourage supplemental milk feeding programs targeted at children on the basis of primary lactose intolerance' (American Academy of Pediatrics, 1990). Low lactose milk has been considered as an alternative to whole milk in the treatment of malnourished children. Studies carried out in the 1970s and 1980s on both animals and humans did not provide evidence that lactose maldigestion impaired the digestion of any other nutrient (Paige and Bayless, 1981; Delmont, 1983).

Lactose is considered to be the preferred carbohydrate for modifying cows' milk for infant formulae. The acid conditions caused by conversion of some lactose to lactic acid in the lower bowel promote the growth of *Bifidobacterium bifidum*, the major microorganism present in the treatment of breast-fed infants. *Bifidobacteria* inhibit the growth of putrefactive bacteria and endow the baby with greater resistance against intestinal infection (Renner, 1983); in addition, they promote synthesis of the B vitamins for absorption.

Lactose maldigestion has been suggested as a risk factor for osteoporosis (Wheadon *et al.*, 1991), because of low calcium intake in susceptible individuals. Low calcium intake has also been implicated in the etiology of other chronic diseases such as hypertension and colon cancer (Miller *et al.*, 1994). Therefore, it is important from a nutritional standpoint to encourage those with lactose maldigestion to develop dietary strategies to include adequate amounts of dairy foods or other sources of these nutrients in their diets. Recommendations include: drinking milk with a meal; consuming servings of one cup or less; trying whole or chocolate milk; trying cheese; consuming yoghurt containing active cultures; using lactose-modified milk products; preparing lactose-reduced milk at home; taking an oral lactase supplement before consuming lactose-containing foods; since non-acceptance of dairy products may be culturally related, including non-dairy foods providing the nutrients of milk in feeding programmes (Miller *et al.*, 1994).

1.8 USES FOR LACTOSE

Lactose is used extensively in the food and pharmaceutical industries, as well as a fermentation substrate for the production of a variety of products.

Since lactose is not as sweet as other commercial sugars, it is exceedingly useful in the processing of many foods. Recent reviews on food uses for lactose are available (Reimerdes, 1990; Zadow, 1991) and many of the comments below are taken from them.

Lactose can be used to increase viscosity or improve texture without making the product too sweet. It has been used in beer manufacture because it is not fermented by the yeast and remains in the finished product to increase viscosity and improve mouth-feel and flavour. It has similar uses in other beverages and foods such as toppings, icings and pie fillings.

The acceptability of milk as a beverage, especially skimmed milk, may be attributed in part to lactose. Its use as a supplement in skimmed milk, buttermilk or chocolate drink improves smoothness and mouth-feel, adds richness and enhances acceptance.

In the meat and sausage industry, lactose may be added to raw sausage formulation as a carbohydrate source for fermentation by selected starter cultures to produce lactic acid for preservation in dry sausage types, such as salami. Lactose also contributes to controlled browning of these products due to Maillard reactions and aids in masking off-flavours and after-tastes caused by emulsifying salts, phosphates and other bitter compounds.

Lactose excels in absorbing flavours, aromas and colourants. It serves as a carrier for flavourings and volatile aromas, and is used to trap such materials during their preparation and in filters to remove undesired volatiles. Lactose may also be used to carry fragrances when a release of odour is needed over a long time period. The binding energies of some typical flavour compounds such as esters, ketones, and aldehydes with lactose range from 5 to 20 kcal mol^{-1}. Lactose is also a carrier for artificial sweeteners such as aspartame and saccharine and can be used as a carrier for colouring agents.

Lactose is added to salad dressing, mayonnaise, soups and sauces to enhance flavour and confer added stability to various proteins in the formulations against flocculation at acid pH and pasteurization. Because of its properties as a free-flow agent, lactose may be added to instant powdered soups and sauces.

The confectionery industry uses lactose in certain types of candies because it changes the crystallization behaviour of the other sugars present and improves body, texture, chewiness and shelf-life. It can be added to fondants at the 20% level to reduce sweetness without affecting other product characteristics.

Lactose is used as an additive to improve the free-flow properties of powdered foods. Lactose glass is used in the instantizing process to

increase the dispersibility of such foods as instantized spray dried milk powder by crystallizing to form agglomerates. It may also be used as an encapsulating agent for volatile flavouring compounds or milkfat or other fats.

In the baking industry, lactose can enhance the creaming properties of shortenings to improve product quality, facilitate baking operations, give increased loaf volume and external appearance score and extend shelf-life, apparently by minimizing starch retrogradation. It can provide better elasticity to the gluten during heating, resulting in better crumb development, crumb stability and pastry volume. In addition, advantage is taken of the ability of lactose to participate in the Maillard reaction which improves browning and flavour of many baked products.

A major use of lactose is in humanized infant formulae where it is used to correct the balance between carbohydrate and protein in breast milk replacers based on cows' milk. Due to its relatively slow digestion, energy is provided to the infant over a period of several hours. Because of its bifidogenic activity, it is viewed as being necessary for the establishment of a healthy intestinal flora. Finally, lactose impacts on mineral absorption, enhancing absorption of calcium, magnesium and possibly zinc (Ziegler and Foman, 1983), perhaps by increasing the permeability of the villus membrane of the intestine.

Lactose has been used for years in the pharmaceutical industry as a coating agent for pills and tablets. A drug may be distributed uniformly in powdered lactose, which is then moulded or compressed into tablets. Other tablets may be given a lactose shell by first wetting the tablet surface with a small amount of a coating syrup, and then tumbling the wetted pill in lactose powder. The coating seals in the contents, but is easily handled and readily dispersible.

The most recent application for lactose and its derivatives is in the formulation of 'nutraceutical' health foods, believed by many to enhance health and well-being, if not actually preventing the development of certain diseases, especially in Japan. Some of these products have been described in earlier sections of this chapter. Further research is needed to gain more information about the effects of long-term consumption of these new foods on maintenance of healthier individuals.

Extensive reviews of lactase-treated dairy products are available (Holsinger, 1978; Zadow, 1984; Mahoney, 1985; Holsinger and Kligerman, 1991; Mitchell, 1991; IDF, 1993); therefore they will not be described further here.

1.8.2 Fermentation

When lactose is used as a substrate for fermentation processes, a wide variety of end-products can be produced. Generally, whey or whey

It has been proposed that fermenting whey anaerobically has the potential to produce calcium magnesium acetate for about the same price as rock salt, $20 to $40 per ton. The compound would be used in place of rock salt as a road deicer as it does not corrode vehicles or harm highway surfaces and surrounding vegetation.

The excellent reviews by Hobman (1984) and Zadow (1984, 1986) may be consulted for further information about some of the products and processes mentioned above. For the future, additional high value products that can be prepared from lactose and hydrolysed lactose need to be sought; examples include new nutraceutical and pharmaceutical products and specialized ingredients (Anonymous, 1990).

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